

## WE CLAIM:

1. A device for determining the isoelectric point of a charged analyte, comprising:
  - a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;
  - an electrode array isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber; and
  - a pH sensor positioned for exposure to liquid introduced into the titration chamber.
2. A device for determining the isoelectric point of a charged analyte, comprising:
  - a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;
  - an electrode array isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber; and
  - a titration reservoir in fluid communication with the titration chamber.
3. A device for determining the isoelectric point of a charged analyte, comprising:
  - a titration chamber comprising an inlet port for introducing a liquid into the titration chamber and an outlet port for exiting a liquid from the titration chamber;
  - an electrode array isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber;
  - an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte in the titration chamber; and
  - a processor operative to receive positional signals from the analyte band detector and to determine the magnitude of position change of an analyte band in the titration chamber in the course of titration.
4. A device for determining the isoelectric point of a charged analyte, comprising:
  - a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;

an electrode array isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber; and

an analyte band detector operative to generate a signal corresponding to detection of a band of analyte downstream of an electric field gradient in the titration chamber.

5. A device for determining the isoelectric point of a charged analyte, comprising:
  - a separation chamber comprising an inlet for introducing a liquid into the separation chamber and an outlet for exiting a liquid from the separation chamber;
  - a first electrode array isolated from the separation chamber and operative to establish an electric field gradient in the separation chamber;
  - a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber; and
  - an electrode array isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber;wherein the separation chamber and the titration chamber are in fluid communication.
6. The device of claim 1, further comprising a second chamber comprising the electrode array.
7. The device of claim 6, wherein the second chamber further comprises an inlet for introducing a liquid and an outlet for exiting a liquid.
8. The device of claim 6, wherein the titration and second chambers are separated by a permeable material.
9. The device of claim 1, further comprising a titration reservoir.
10. The device of claim 1, further comprising an analyte band detector.
11. The device of claim 10, wherein the analyte band detector is in the first chamber.

12. The device of claim 1, further comprising molecular sieve in the titration chamber to shift the location of a stationary focused band of charged analyte in the titration chamber for a given set of focusing conditions.
13. The device of claim 5, further comprising molecular sieve in the separation chamber to shift the location of a stationary focused band of charged analyte in the separation chamber for a given set of focusing conditions.
14. The device of claim 1, wherein the pH sensor comprises an ion selective electrode.
15. The device of claim 3, wherein the analyte band detector comprises a potentiometric detector.
16. The device of claim 3, wherein the analyte band detector comprises UV-Visible spectroscopy detector.
17. The device of claim 1, further comprising a mixing chamber with an inlet for receiving a liquid, an inlet for receiving a titrating solution, and an outlet connected to the inlet of the first chamber.
18. The device of claim 17, wherein the mixing chamber comprises a static mixer.
19. The device of claim 1, further comprising a dialyzer for dialyzing ions from a titrating solution to a flowing liquid or from a flowing liquid to a titrating solution.
20. A method of determining the isoelectric point of a charged analyte comprising:
  - focusing a charged analyte in a flowing liquid in an electric field gradient to form in the flowing liquid a focused band of the charged analyte at a stable position in the electric field gradient;
  - incrementing the pH of the flowing liquid at least once by an amount sufficient to change the position of the focused band of the charged analyte within the electric field gradient;

obtaining pH and corresponding position data for the charged analyte, comprising determining the pH of the flowing liquid and the corresponding position of the focused band of the charged analyte at a plurality of band positions within the electric field gradient; and

determining the isoelectric point of the charged analyte based on the pH and corresponding position data.

21. The method of claim 20, wherein the isoelectric point is determined by extrapolation.
22. The method of claim 20, wherein the pH is incremented to a plurality of pH's above the isoelectric point of the charged analyte and to a plurality of pH's below the isoelectric point of the charged analyte.
23. The method of claim 22, wherein the isoelectric point is determined by interpolation.
24. The method of claim 20, wherein the pH of the flowing liquid is incremented by mixing the flowing liquid with a titrating solution.
25. The method of claim 20, wherein the pH of the flowing liquid is incremented by dialyzing ions from a titrating solution into the flowing liquid.
26. The method of claim 22, wherein  
the pH is incremented from above the isoelectric point downward until an upper bracketing pH at which the charged analyte elutes is reached;  
the pH is incremented from below the isoelectric point upward until a lower bracketing pH at which the charged analyte elutes;  
the upper bracketing pH and the lower bracketing pH are obtained; and  
the isoelectric point is determined by averaging the upper bracketing pH and the lower bracketing pH.
27. The method of claim 20, wherein the charged analyte is first focused in a DFGF chamber.

28. The method of claim 27, wherein the DFGF chamber comprises a separation chamber which comprises molecular sieve operative to shift the location at which each stationary focused band of charged analyte forms under the focusing process parameters.
29. The method of claim 20, wherein the charged analyte comprises a biomacromolecules.
30. The method of claim 29, wherein the biomacromolecules comprises protein.
31. The method of claim 29, wherein the biomacromolecules comprises DNA.
32. The method of claim 20, wherein the charged analyte comprises multiple charged analytes.
33. The method of claim 26, wherein an analyte band detector is used to sense elution of the charged analyte.
34. The method of claim 26, wherein the sample is split and portions of the sample are separately focused and incremented.
35. The method of claim 20, wherein the pH is determined by calculation from mixing a known amount of a titrating solution of known pH with a known amount of a flowing liquid of known pH.
36. A method of developing a two-dimensional display of information regarding a charged analyte, comprising:  
determining the molecular weight of the charged analyte;  
determining the isoelectric point of the charged analyte; and  
displaying the molecular weight and the isoelectric point in a two-dimensional display format comprising a first axis representing molecular weight values and a second axis representing isoelectric point values;  
wherein determining the isoelectric point of the charged analyte comprises

focusing a charged analyte in a flowing liquid in an electric field gradient to form in the flowing liquid a focused band of the charged analyte at a position in the electric field gradient;

incrementing the pH of the flowing liquid at least once by an amount sufficient to change the position of the focused band of the charged analyte within the electric field gradient;

obtaining pH and corresponding position data for the charged analyte, comprising determining the pH of the flowing liquid and the corresponding position of the focused band of the charged analyte at a plurality of band positions within the electric field gradient; and  
determining the isoelectric point of the charged analyte based on the pH and corresponding position data.

37. The method of claim 36, wherein the two-dimensional display is generated by a computer and displayed on a monitor.

38. A method of developing a two-dimensional display of information regarding charged analytes, comprising:

determining the molecular weight of each of multiple charged analytes;

determining the isoelectric point of each of the charged analytes; and

displaying the molecular weight and the isoelectric point for each of the charged analytes in a two-dimensional display format comprising a first axis representing molecular weight values and a second axis representing isoelectric point values;

wherein determining the molecular weight of each of the charged analytes comprises:

providing a device for separating and focusing the charged analytes comprising:

a first chamber comprising an inlet for introducing liquid into the first chamber and an outlet for exiting liquid from the first chamber; and  
an electrode array isolated from the first chamber and operative to be energized to establish an electric field gradient in the first chamber;

establishing a flow of liquid comprising the multiple charged analytes through the first chamber under a set of focusing process parameters including an

electric field gradient sufficient to focus the multiple charged analytes each in a corresponding stationary focused band in the electric field gradient;  
wherein the first chamber contains molecular sieve operative to shift the location at which each stationary focused band of charged analyte forms under the focusing process parameters;  
and wherein determining the isoelectric point of the charged analytes comprises:

focusing each of the charged analytes in a flowing liquid in an electric field gradient to form in the flowing liquid a stationary focused band of the charged analyte at a position in the electric field gradient;

incrementing the pH of the flowing liquid at least once by an amount sufficient to change the position of the stationary focused band of the charged analyte within the electric field gradient;

obtaining pH and corresponding position data for the charged analyte, comprising determining the pH of the flowing liquid and the corresponding position of the focused band of the charged analyte at a plurality of band positions within the electric field gradient; and

determining the isoelectric point of the charged analyte based on the pH and corresponding position data.

39. The method of claim 20, wherein the charged analyte is first focused in an EFGF chamber.

40. The method of claim 39, wherein the EFGF chamber comprises a separation chamber which comprises molecular sieve operative to shift the location at which each stationary focused band of charged analyte forms under the focusing process parameters.

41. The method of claim 39, wherein the EFGF chamber comprises a configured electrode chamber.

42. The method of claim 39, wherein the EFGF chamber comprises a configured separation chamber.

43. A device for determining the isoelectric point of a charged analyte, comprising:  
a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;  
electrodes isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber; and  
a pH sensor positioned for exposure to liquid introduced into the titration chamber;  
wherein the titration chamber has a non-uniform cross-section flow channel; and  
wherein the electrodes comprise at least two electrodes.
44. The device of claim 43, further comprising an electrode chamber comprising the electrodes.
45. The device of claim 44, wherein the titration and electrode chambers are separated by a permeable material.
46. The device of claim 44, wherein the electrode chamber has a non-uniform cross-section flow channel.
47. A device for determining the isoelectric point of a charged analyte, comprising:  
a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;  
electrodes isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber; and  
a titration reservoir in fluid communication with the titration chamber;  
wherein the titration chamber has a non-uniform cross-section flow channel; and  
wherein the electrodes comprise at least two electrodes.
48. The device of claim 47, further comprising an electrode chamber comprising the electrodes.
49. The device of claim 48, wherein the titration and electrode chambers are separated by a permeable material.



50. The device of claim 48, wherein the electrode chamber has a non-uniform cross-section flow channel.
51. A device for determining the isoelectric point of a charged analyte, comprising:  
a titration chamber comprising an inlet port for introducing a liquid into the titration chamber and an outlet port for exiting a liquid from the titration chamber;  
electrodes isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber;  
an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte in the titration chamber; and  
a processor operative to receive positional signals from the analyte band detector and to determine the magnitude of position change of an analyte band in the titration chamber in the course of titration;  
wherein the titration chamber has a non-uniform cross-section flow channel; and  
wherein the electrodes comprise at least two electrodes.
52. The device of claim 51, further comprising an electrode chamber comprising the electrodes.
53. The device of claim 52, wherein the titration and electrode chambers are separated by a permeable material.
54. The device of claim 52, wherein the electrode chamber has a non-uniform cross-section flow channel.
55. A device for determining the isoelectric point of a charged analyte, comprising:  
a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;  
electrodes isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber; and  
an analyte band detector operative to generate a signal corresponding to detection of a band of analyte downstream of an electric field gradient in the titration chamber;  
wherein the titration chamber has a non-uniform cross-section flow channel; and  
wherein the electrodes comprise at least two electrodes.

56. The device of claim 55, further comprising an electrode chamber comprising the electrodes.
57. The device of claim 56, wherein the titration and electrode chambers are separated by a permeable material.
58. The device of claim 56, wherein the electrode chamber has a non-uniform cross-section flow channel.
59. A device for determining the isoelectric point of a charged analyte, comprising:  
a separation chamber comprising an inlet for introducing a liquid into the separation chamber and an outlet for exiting a liquid from the separation chamber;  
first electrodes isolated from the separation chamber and operative to establish an electric field gradient in the separation chamber;  
a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber; and  
second electrodes isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber;  
wherein the separation chamber and the titration chamber are in fluid communication;  
wherein the titration chamber has a non-uniform cross-section flow channel; and  
wherein the first electrodes and the second electrodes each comprise at least two electrodes.
60. The device of claim 59, further comprising a first electrode chamber comprising the first electrodes.
61. The device of claim 59, further comprising a second electrode chamber comprising the second electrodes.
62. The device of claim 60, wherein the separation and first electrode chambers are separated by a permeable material.
63. The device of claim 61, wherein the titration and second electrode chambers are separated by a permeable material.

64. The device of claim 60, wherein the first electrode chamber has a non-uniform cross-section flow channel.
65. The device of claim 61, wherein the second electrode chamber has a non-uniform cross-section flow channel.
66. The device of claim 60, wherein the separation chamber has a non-uniform cross-section flow channel.
67. The device of claim 60, wherein the first electrodes comprise an electrode array.
68. A device for determining the isoelectric point of a charged analyte, comprising:  
a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;  
an electrode chamber that has a substantially uniform depth and a non-uniform width; electrodes in the electrode chamber operative to establish an electric field gradient in the titration chamber; and  
a pH sensor positioned for exposure to liquid introduced into the titration chamber;  
wherein the electrodes comprise at least two electrodes; and  
wherein the electrode chamber has a substantially uniform depth and a non-uniform width.
69. The device of claim 68, wherein the titration and electrode chambers are separated by a permeable material.
70. The device of claim 68, wherein the titration chamber has a uniform cross-section flow channel.
71. The device of claim 69, wherein the titration chamber has a non-uniform cross-section flow channel.
72. A device for determining the isoelectric point of a charged analyte, comprising:

a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;  
an electrode chamber that has a substantially uniform depth and a non-uniform width;  
electrodes in the electrode chamber operative to establish an electric field gradient in the titration chamber; and  
a titration reservoir in fluid communication with the titration chamber;  
wherein the electrodes comprise at least two electrodes; and  
wherein the electrode chamber has a substantially uniform depth and a non-uniform width.

73. The device of claim 72, wherein the titration and electrode chambers are separated by a permeable material.

74. The device of claim 72, wherein the titration chamber has a uniform cross-section flow channel.

75. The device of claim 73, wherein the titration chamber has a non-uniform cross-section flow channel.

76. A device for determining the isoelectric point of a charged analyte, comprising:  
a titration chamber comprising an inlet port for introducing a liquid into the titration chamber and an outlet port for exiting a liquid from the titration chamber;  
an electrode chamber that has a substantially uniform depth and a non-uniform width;  
electrodes in the electrode chamber operative to establish an electric field gradient in the titration chamber;  
an analyte band detector operative to generate positional signals corresponding to positions of a band of analyte in the titration chamber; and  
a processor operative to receive positional signals from the analyte band detector and to determine the magnitude of position change of an analyte band in the titration chamber in the course of titration;  
wherein the electrodes comprise at least two electrodes; and

wherein the electrode chamber has a substantially uniform depth and a non-uniform width.

77. The device of claim 76, wherein the titration and electrode chambers are separated by a permeable material.
78. The device of claim 76, wherein the titration chamber has a uniform cross-section flow channel.
79. The device of claim 77, wherein the titration chamber has a non-uniform cross-section flow channel.
80. A device for determining the isoelectric point of a charged analyte, comprising:  
a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber;  
an electrode chamber that has a substantially uniform depth and a non-uniform width;  
electrodes in the electrode chamber operative to establish an electric field gradient in the titration chamber; and  
an analyte band detector operative to generate a signal corresponding to detection of a band of analyte downstream of an electric field gradient in the titration chamber;  
wherein the electrodes comprise at least two electrodes; and  
wherein the electrode chamber has a substantially uniform depth and a non-uniform width.
81. The device of claim 80, wherein the titration and electrode chambers are separated by a permeable material.
82. The device of claim 80, wherein the titration chamber has a uniform cross-section flow channel.
83. The device of claim 81, wherein the titration chamber has a non-uniform cross-section flow channel.

84. A device for determining the isoelectric point of a charged analyte, comprising:  
a separation chamber comprising an inlet for introducing a liquid into the separation chamber and an outlet for exiting a liquid from the separation chamber;  
a first electrode chamber that has a substantially uniform depth and a non-uniform width;  
first electrodes in the first electrode chamber operative to establish an electric field gradient in the titration chamber;  
a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber; and  
second electrodes isolated from the titration chamber and operative to establish an electric field gradient in the titration chamber;  
wherein the separation chamber and the titration chamber are in fluid communication;  
wherein the first electrode chamber has a non-uniform cross-section flow channel;  
and  
wherein the first electrodes and the second electrodes each comprise at least two electrodes.
85. The device of claim 84, further comprising a second electrode chamber comprising the second electrodes.
86. The device of claim 84, wherein the separation and first electrode chambers are separated by a permeable material.
87. The device of claim 85, wherein the titration and second electrode chambers are separated by a permeable material.
88. The device of claim 85, wherein the second electrode chamber has a non-uniform cross-section flow channel.
89. The device of claim 84, wherein the separation chamber has a non-uniform cross-section flow channel.

90. The device of claim 84, wherein at least one of the first electrodes and second electrodes comprise an electrode array.
91. A device for determining the isoelectric point of a charged analyte, comprising:  
a separation chamber comprising an inlet for introducing a liquid into the separation chamber and an outlet for exiting a liquid from the separation chamber;  
first electrodes isolated from the separation chamber and operative to establish an electric field gradient in the separation chamber;  
a titration chamber comprising an inlet for introducing a liquid into the titration chamber and an outlet for exiting a liquid from the titration chamber; and  
a titration electrode chamber that has a substantially uniform depth and a non-uniform width;  
second electrodes in the titration electrode chamber operative to establish an electric field gradient in the titration chamber;  
wherein the separation chamber and the titration chamber are in fluid communication;  
wherein the titration electrode chamber has a non-uniform cross-section flow channel; and  
wherein the first electrodes and the second electrodes each comprise at least two electrodes.
92. The device of claim 90, further comprising a first electrode chamber comprising the first electrodes.
93. The device of claim 90, wherein the titration chamber and titration electrode chamber are separated by a permeable material.
94. The device of claim 91, wherein the separation and first electrode chambers are separated by a permeable material.
95. The device of claim 91, wherein the first electrode chamber has a non-uniform cross-section flow channel.

96. The device of claim 90, wherein the separation chamber has a non-uniform cross-section flow channel.

97. The device of claim 90, wherein the titration chamber has a non-uniform cross-section flow channel.

98. The device of claim 90, wherein at least one of the first electrodes and second electrodes comprise an electrode array.